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Synthetic Lethal Gene for PTEN as a Therapeutic Target

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14. ABSTRACT

PTEN is the gene which is down-regulated in the majority of prostate cancer, and PTEN-Akt pathway is considered to play a critical role in radiation resistance. The overall goal of this project is to identify a specific gene that is essential for this important pathway in the hope that targeting such gene will sensitize PTEN-negative tumor stem cells to be radiation-sensitive. To accomplish this goal, we isolated cancer stem cells from prostate tumor cell lines and patient samples followed by preparing PTEN-deficient cells. We then screened genes that play critical roles in the PTEN pathway using a technique called the shRNA library screening, with or without radiation treatment of these cells. We have identified "synthetic lethal" genes, and we plan to examine the effect of such genes in an animal model to test our hypothesis. During the funding period, we have successfully accomplished Aim 1 and 2 and we identified five synthetic lethal genes. Testing the effect of knockout of these genes on radiation therapy in an *in vivo* model as planed in Aim 3 is currently in progress. We believe that targeting the synthetic lethal genes that we discovered will lead to the development of a novel therapeutic approach for radiation-resistant prostate cancer.

15. SUBJECT TERMS

Prostate cancer, tumor stem cells, PTEN, resistance

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INTRODUCTION

Prostate cancer is the most frequently diagnosed cancer and the second leading cause of cancer death among men in the US. Down-regulation of tumor suppressor gene, PTEN, has been found in up to 60% of advanced prostate cancer, and therefore, PTEN is considered to play a critical role in tumor progression of prostate cancer (1-3). PTEN is a phosphatase that antagonizes the phosphoinositol-3-kinase/AKT signaling pathway and suppresses cell survival as well as cell proliferation. PTEN is also known to suppress self-renewal of CSC which is believed to be responsible for chemo-resistance. Therefore, down-regulation of PTEN and concomitant activation of AKT pathway endows tumor cells with survival advantage during chemo- and radiation-therapy (4). We hypothesize that there are synthetic lethal genes that are up-regulated when PTEN function is lost in prostate cancer. Knock-down of such gene in PTEN-negative CSC is expected to be lethal when they are treated with radiation. The purpose of this project is to identify such gene(s) by particularly focusing on kinase genes. We expect that such gene will be an excellent therapeutic target to overcome resistance to chemo-, radiation and hormone-therapy for prostate cancer patients. The main objective of this project is to identify synthetic lethal gene(s) in PTEN-negative prostate CSC.

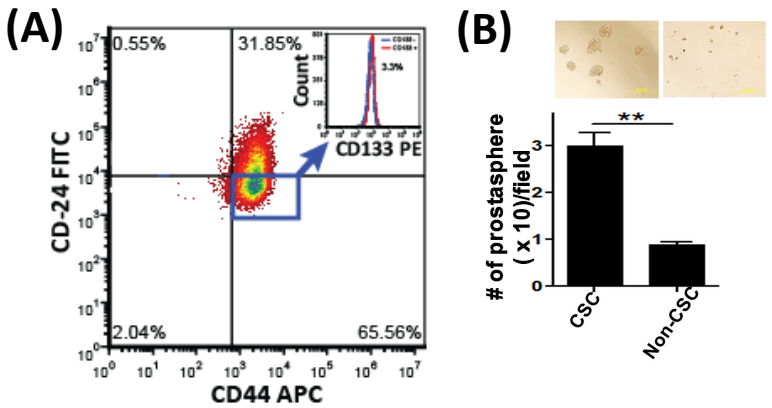
BODY

The main objective of this project is to identify synthetic lethal gene(s) in PTEN-negative prostate CSC. To test our hypothesis, we will first isolate CSC from patients and prepare PTEN-knockdown cells (**Aim 1**). We will then a screen a shRNA library for synthetic lethal genes in these cells with or without radiation treatment (**Aim 2**). When we identify a synthetic lethal gene, we will then test the effect of such gene in an animal model (**Aim 3**).

Aim 1. To isolate CSC from human cell lines and patient samples and prepare PTEN-knockdown cells.

Progress

We have isolated CSCs population using $CD24^{low}/CD44^{high}/CD133^{high}$ from various prostate cancer cell lines including PC3mm and C4-2B. Our initial FACS analysis indicates that PC3mm has around 3% of stem cell population (Fig. 1A). When they are cultured in serum-free medium, they generated significantly higher numbers of large prostatspheres compared to non-stem cells (Fig. 1B). We also transplanted the CSCs prepared from PC3mm into nude mice and found that they have significantly stronger abilities to initiate tumorigenesis compared to the original cells as shown in Fig. 1C.



# of Cells	10^4	10^3	10^2
Stem ($CD24^-/CD44^+/CD133^+$)	5/6	3/6	1/6
Non-Stem	2/6	1/6	0/6

Fig. 1. Preparation of cancer stem cells. (**A**) Tumor stem cell population in PC3mm was analyzed by FACS using specific antibodies to CD24, CD44, and CD133. (**B**) Prostasphere formation of CSCs. (**C**) Tumor stem cells isolated from PC3mm were injected subcutaneously into nude mice for limiting dilution analysis, and the growth of tumor was monitored by Xenogen bioimaging system.

We obtained the similar results for CSCs from C4-2B cells. We purchased the shRNA expressing lentiviral vector (Open-Bioscience) against the PTEN gene and prepared high-titer lentiviruses. We then infected them to PC3mm and C2-4B cell lines followed by establishing cells by puromycine screening. These cell lines are designated as PC3mm-shPTEN and C24B-shPTEN, and they were used in Specific aim 2.

We tried to isolate CSCs from clinical samples; however, it was not successful. One of the major reasons is a small size of the samples and yield was very low for the next step of experiments. Therefore, we decided to use the "processed" tissues that were obtained from Conversant. They collect prostate cancer tissues and processed to single cells followed by directly transplantation into SCID mice to grow tumors without cell culture. We obtained cell suspension and sorted the cells using the surface marker, $CD24^{low}/CD44^{high}/CD133^{high}$, by using the MACS system. This approach successfully yielded more than 10^4 CSCs. The cells were passaged through SCID mice two times to amplify them and their single cell suspension was infected with shRNA-PTEN lentivirus. These cells are used for Specific aim 2 experiment. Therefore, Aim 1 is completed.

Aim 2. To screen shRNA library for synthetic lethal genes in these cells with or without radiation treatment.

Progress

We obtained multiple shRNA libraries from Addgene. These include the DECIPHER Lentiviral shRNA Library with barcodes and they are targeted to pathway, disease, and cell surface, respectively. We prepared the pathway-targeted lentivirus library and tested for the titers of each preparation. We then infected the virus libraries to CSCs that were prepared from PC3mm and the clinical sample. After screening these cells by red-fluorescent signal in the sphere medium by FACS, we established cell lines that express shRNA libraries in CSCs of PC3mm and the clinical sample. Therefore, we have established 6 sets of lines. To examine the complexity of the library, we pulled out 50 individual clones and measured the expression of 20 different kinases. We found that 5 kinases were significantly lowed (knocked down), and the expression of the rest of the 15 genes were unchanged. The distribution of five kinases was not in a random fashion. Accordingly, we abandoned these libraries.

We then obtained the siRNA library of human Kinase genes from Sigma Co (Mission library). This library consists of 719 kinase genes and individually spotted in 96-well plates. To screen this library, we first irradiated CSCs that were prepared from PC3mm with or without PTEN knockdown at 0.25 Gy followed by distributing these cells into the 96-well plates of the kinase library. The plates were incubated at 37C with 5% CO2 for 4 days. The plates were then measure for red florescent signals for the survival of CSCs in each well to identify siRNA that killed PTEN- but not PTEN+ cells. This screening resulted in 5 known kinases (PI3K, Akt, p38, GSK3, MEK1/2). These genes are considered to be synthetic lethal genes for PTEN, and they are likely to serve as targets for prostate tumor with PTEN-negative and radiation resistant. Therefore, the Aim 2 is accomplished.

Aim 3. When we identify a synthetic lethal gene, we will then test the effect of such gene in an animal model

Progress

To accomplish Aim 3, we have constructed PC3mm-shPTEN with or without knock-down of 5 kinase genes that were identified in Aim 2. Cells were screened by GFP and we have established these cell

lines. All these cells were viable and they were secured as a frozen stock. Knockdown of each gene was confirmed by Western blot assay. We plan to test the effect of the knockdown of these synthetic lethal genes by first orthotropically transplanting them into nude mice followed by treating them with irradiation at 2Gy at the lower abdomen for three times followed by monitoring the tumor growth. We expect that mice that are implanted with $PTEN^-$ and the shRNA to these 5 genes will be more responsive to the radiation treatment. This experiment is in progress.

KEY RESEARCH ACCOMPLISHMENTS

1. We were able to isolate CSCs from multiple prostate cancer cell lines and also from clinical samples. They were validated for their tumor initiating abilities *in vivo*.
2. We have established PTEN knockdown cell lines of prostate cancer.
3. We have successfully prepared a siRNA library in these cell lines and screened for synthetic lethal genes for PTEN.
4. We identified 5 genes as the synthetic lethal genes of PTEN and these include PI3K, Akt, p38, GSK3, MEK1/2.
5. We have established cell lines with knock-down of these genes in PC3mm-shPTEN, that are ready for *in vivo* experiment for Aim 3.

REPORTABLE OUTCOMES

Peer reviewed publications
None.

Employment
1. Ms.Yin Liu (Graduate student) has been partly supported by the current grant.

CONCLUSIONS

This project has been delayed due to the relocation of our entire lab to University of Mississippi Medical Center, and we needed to re-establish our lab setting including personnel. However, we successfully established necessary cell lines and isolating CSCs as well as completing siRNA library screening. We discovered five genes (PI3K, Akt, p38, GSK3, MEK1/2) as synthetic lethal genes for PTEN. These genes are considered as potential targets for $PTEN^-$ and radiation-resistant prostate cancer. Aim 1 and 2 were accomplished, and Aim3 experiment is underway which will further verify our overall hypothesis.

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